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Isolation and characterization of soil bacteria in the vicinity of brick field under air pollution condition and their potential role as plant growth promoter

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ABSTRACT:
The excessive air pollution has tremendous effect on soil borne microorganisms as well as it affects the soil fertility in greater extent. The continuous exposure of these air pollutants in agricultural crops often lead to reduction of soil fertility and it has a tremendous deleterious effect on annual crop yield also. The industrial development as well as the aerial pollutants are also one of the most responsible cause of decreasing of crop yield. The dense aerial pollutant gases emitted from brick field also has dangerous effect on crop yield. In this article we are going to focus on the potential role of soil bacteria having plant growth promoting activities, isolated from the agricultural land situated in vicinity of brick field .We examined the growth of spinach plant with four isolated soil bacterial strains under exposure of air pollutant gases near the brick field which is situated just beside of 34 no. National high way. In all studied cases, we observed that different growth parameters in the uninoculated spinach *(Spinacia oleracea L.)* plants (i.e-without soil bacteria) were reduced as compared with treated plants. Air pollutant toxicity were assessed by observing different growth parameters like reduced growth and weak rooting and low chlorophyll content. Inoculation of soil bacteria in spinach plant reduced the deleterious effects of air pollution to some extent and enhanced the different growth parameters as compared with un inoculated aerially polluted plants. Our present study conducted with 4 out of 25 phenotypically and genotypically different bacterial isolates and observed results indicated that SIC 1,SIC 2,SIC3 ,SIC4 (named according to the place and priority of isolate such as, Sample Isolate Chakdaha 1) were very effective to promote plant growth.

Key words: Air pollution stress, soil bacteria, *Spinacia oleracea L. , reduced growth, weak rooting.*

INTRODUCTION:

In today's agricultural system the continuous application of chemical fertilizers specially the nitrogenous and phosphorus has led to tremendous deleterious effect not only on soil fertility but also responsible for polluting the air, water and soil environment. They are also affecting the activities of soil microorganisms [1].The term 'rhizosphere' or root surrounding area was first introduced by Hiltner [2].The rhizospheric soil microorganisms has great influence to guard the plant in the eco-friendly manner [3].They take part in several biotic activities in soil ecosystem and make possible the sustainable crop production[4].The rhizospheric zone constitute several bacteria and fungi which have beneficial, neutral as well as delirious effects on plants [5]. Some of them can be growth improver of plants [6,7].There are lots of reports of beneficial soil microorganisms on growth of plant and yield stimulation[8,9]. There are several air pollutants in the atmosphere but specifically ozone (O_3),carbon di oxide (CO_2) and other green house gases are emitting in the environment. Among these ozone is secondary pollutant and it is synthesized by the atmospheric photochemical reactions that involves oxides of nitrogen and reactive hydrocarbons emitted from running automobiles [10]. Ozone is a dangerous phytotoxic air pollutant which reduces plant productivity tremendously [11].

In this study the preliminary objective is to isolate soil bacteria from the rhizospheric soil of agricultural field from brick field vicinity, near the 34 no. National highway , Chakdaha (where air pollution is in high range) and to estimate the effects of those bacterial strains on early seedling growth of spinach plants. In addition to that the chlorophyll content of the young plants were also measured.

MATERIALS AND METHODS:

Collection of soil:

Soil samples were collected from agricultural research plots of vegetable (mainly cabbage during late winter season) and paddy (during early summer season) adjoining to 34th National highway of Chakdaha (Nadia) surrounded by few industries. During collection upper 5 cm. soils were discarded

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and the soil layers beyond this were collected aseptically in clean polythene bag, brought to the laboratory and stored in aseptic condition as far as possible for further use.

Collection of spinach seeds:
Spinach seeds (Harita variety) of uniform germplasm were collected from Uluberia Seed Bank. After purchase seeds were stored in dried places of laboratory for further use.

Isolation of bacteria:
Bacterial strains from collected soil samples were isolated by serial dilution technique employing media such as Nutrient Agar Media (Beef extract-3g; Peptone-5g; Agar-5g; Distilled water-1000ml., Sodium chloride- 5g, p^H-6.8). Plates were incubated at 28^0c and after 48-72 hours of incubation the number of bacterial colonies developed was recorded and inoculated separately in Nutrient Agar slants, maintained at 4^0c for further work.

Characterisation of bacteria:
The isolated bacterial strains were characterized by their morphological, cultural, staining and biochemical properties.

Morphological characters include colour, elevation and edge of the colony, presence or absence of capsule of individual isolates.

Gram nature of each isolates was initially determined by using crystal violet and safranin staining. Among biochemical tests, amylase test, catalase test, gelatin hydrolysis were performed based on conventional techniques for bacterial characterization. (followed by Microbiology- A Laboratory Manual, 7th edition by Capuccino Sherman).

Seed germination test:
The efficiency of germination of the collected spinach seeds were performed. Atfirst the spinach seeds were surface sterilized with 0.01% $Hgcl_2$ for 2 mins. followed by successive washing with sterile distilled water. Seeds were kept into respective bacterial culture medium containing 10^6 cells/ml. for 10 mins. After that the seeds were transferred and placed on sterile soil containing pots and incubated for 2-3 days. After 3 days seed germination was recorded in comparision with control. The nature of seed germination was also checked by planting the imbibed seeds into pots using the same technique.

Germination percentage = (Total no. of germinated seeds/Total no. seeds)*100

Exploitation of soil bacteria on growth of spinach seedlings in air pollution stress condition:
Spinach seeds (Harita variety) were sown in sterilized for successive 3 days at 15 lbs pressure for 40 mins.) in pot culture after proper imbibitions in the bacterial suspension for 24 hours and the set was kept in aerially polluted zone in Chakdaha adjacent to 34 no. National high way. After 14 days of sowing plantlets were carefully uprooted and brought to the laboratory. The length of root, shoot and chlorophyll content of the test plant samples were measured.For chlorophyll content measurement of green parts of spinach plants the conventional method was followed (the method of Arnon, D I (1949) Plant Physiol 241)

RESULTS AND DISCUSSION:

In this study soil bacteria from polluted area were isolated and enumerated their role for improve the growth and yield of spinach in the same environmental condition beside the 34th National highway where frequently automobile discharged gases were emitted due to running of heavy vehicles throughout the day. Usually the nature possess different types of gases like oxygen, carbon di oxide, carbon monoxide etc. Other gaseous substances such as ozone which involves the oxides of nitrogen and reactive hydrocarbons when persisted in nature in various amount then the condition become intolerant to the plants and it had profound effects on plant growth and eventually the yield also(10).

Colony morphology and other biochemical tests of the isolates were performed following the standard methods of " Microbiology - A Laboratory Manual" by Cappuccino Sherman(7th edition). It was noted that presence of yellow colouration, bubble production, liquefaction of gelatin were indicated as depicted in Table 1.

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Exploitation of soil bacteria on spinach seedling under air pollution stress condition:

Effect of soil bacteria on seed germination under aerially polluted environment was estimated. The result indicated that the role of bacterial isolates had tremendous effects on the germination of spinach seeds. It was further noted that the set was at Chakdaha over a building's roof which was exposed towards 34no. National highway and the building was in front of a brick field.

The surface sterilized seeds were soaked with different combinations of bacterial suspensions for 24 hours and then allowed them to germinate on the pots kept in Chakdaha (the aerially polluted zone) and after the stipulated period the germination percentage was calculated as caompared to uninoculated control set (depicted in Fig 1)

Table1: Isolation and characterization of bacteria:

Name of the strain	Colony Morphology	Gram nature	Amylase Test	Catalase Test	Gelatin Hydrolysis
SIC1	Whitish, smooth	+	++	+	-
SIC2	Whitish, smooth	+	+	++	-
SIC3	Yellowish, elevated	+	+++	+	-
SIC4	Slightly yellowish, elevated	+	+	+	+

** Presence or absence of character is denoted by "+" or "-" sign; intensity of character is denoted by the no. of "+" sign.

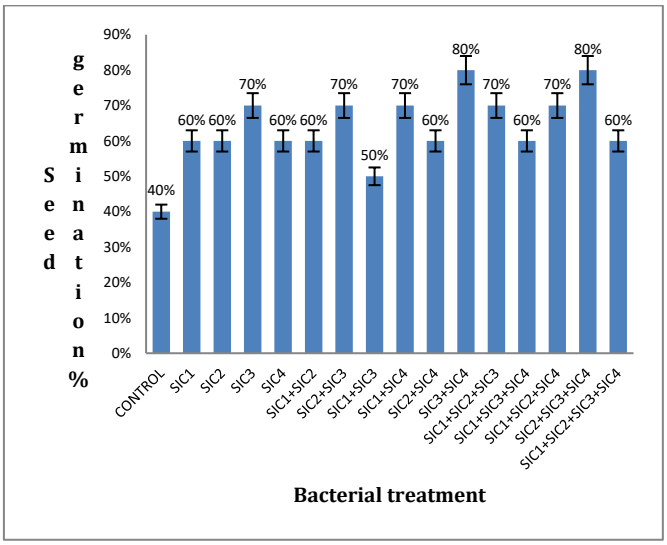

Fig 1: Effect of isolated bacterial strains on seed germination of spinach at Chakdaha.

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Table 2: Effects of soil bacteria on growth under aerially polluted zone of Chakdaha:

EXPERIMENTAL SETS	TOTAL NO. OF SEEDS USED	NO. OF SEEDS GERMINATED	ENHANCEMENT OF LENGTH (cm.)		CHLOROPHYLL CONTENT (mg/gm of tissue)
			Shoot length	Root length	
CONTROL	20	8	2.7	1.1	6.70
SIC1	20	12	3.3	1.6	7.76
SIC2	20	12	3	1.4	14.48
SIC3	20	14	3.9	2.4	17.87
SIC4	20	12	3.9	1.3	16.10
SIC1+SIC2	20	12	2.9	1.9	17.49
SIC2+SIC3	20	14	4.6	2.8	15.38
SIC1+SIC3	20	10	3.9	2.2	7.88
SIC1+SIC4	20	14	3.6	2.7	24.56
SIC2+SIC4	20	12	4.2	2.7	7.60
SIC3+SIC4	20	16	4.3	1.6	17.93
SIC1+SIC2+SIC3	20	14	3.1	2.6	22.14
SIC1+SIC3+SIC4	20	12	4.3	1.9	26.30
SIC1+SIC2+SIC4	20	14	4.6	1.9	17.89
SIC2+SIC3+SIC4	20	16	2.9	1.5	12.00
SIC1+SIC2+SIC3+SIC4	20	12	3.7	1.6	16.73

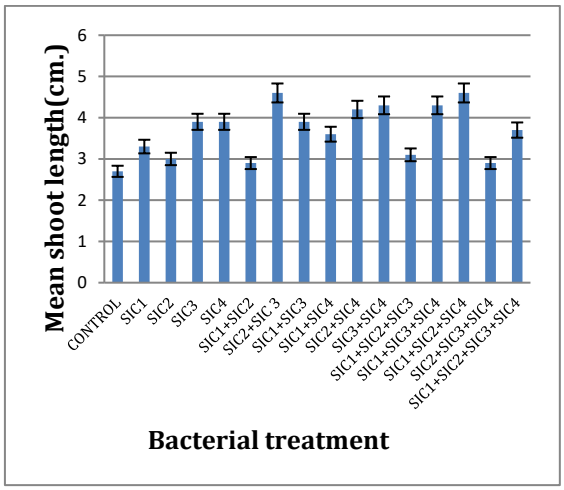

FIG 2 : Effect of isolated bacterial strains on shoot length(cm.) of spinach seedlings at Chakdaha.

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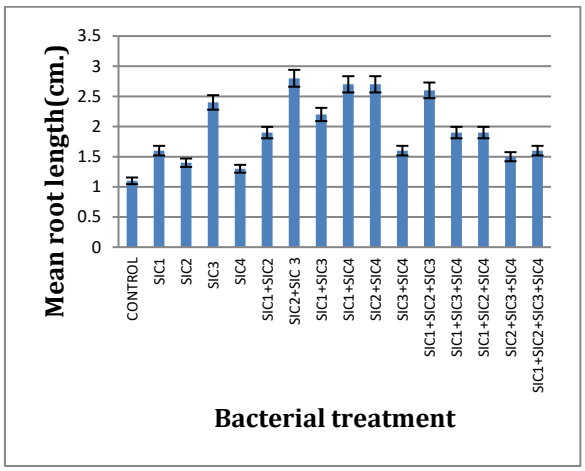

FIG 3 :
Effect of isolated bacterial strains on root length(cm.) of spinach seedlings at Chakdaha.

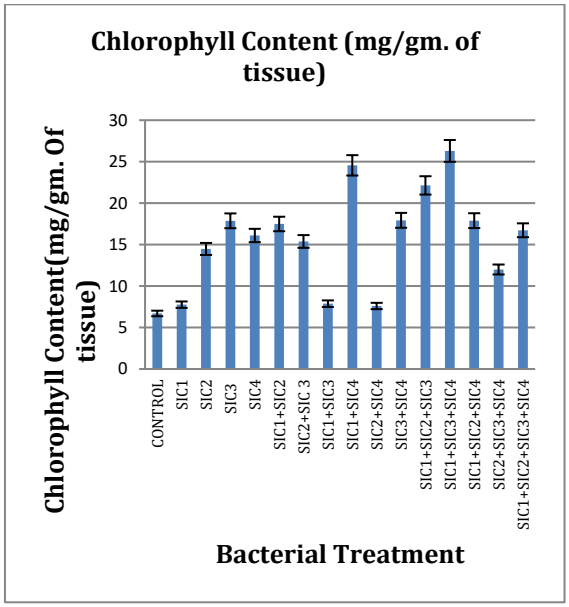

FIG 4. Effect of isolated bacterial strains on chlorophyll content (mg/gm.) of spinach seedlings at Chakdaha. (followed by the method of Arnon, D I (1949) Plant Physiol 241)

The outcome of the experiment indicated that the isolated bacterial strains had some remarkable effects on seed germination under aerially polluted condition. So, from this experiment we can conclude that these soil bacterial strains can be used as seed germination enhancer in aerially polluted condition. The bacterial isolates were characterized by morphological studies followed by determination of gram nature and their biochemical properties as depicted in results following the standard method (12) (table 1). The results indicated that the bacteria possess different biochemical characteristics.

In a subsequent study the bacteria were used as inoculants and enumerate their effects by measuring root length (fig 2), shoot length(fig 3) and chlorophyll content (fig 4) of test plant by using various combinations of the isolated bacteria as compared to uninoculated set. The result completely represented in table 2. The result

clearly indicated that when the bacteria were used as inoculants the growth and ultimately the yield of spinach plants were remarkably improved which indicated that the inoculated soil bacteria act as plant growth promoter and eventually had profound role on improved growth of spinach plants.

Information about soil borne plant promoting bacteria were many and their precise role for growth promotion were also evaluated in different plants, such as, rice (13), wheat (14), cucumber (15), maize (16), cotton(17), black pepper (18) and banana (19). The results obtained in this study therefore, in confirmation of the previous results.

Furthermore, in this study the bacteria were isolated from soil of aerially polluted zone and the entire experiments were carried out in the same environmental condition. Thus, the results obtained in this study clearly indicated that soil bacteria promote to overcome the adverse effects of pollutant gases and which is perhaps reported first time in this report. Extensive study need for draw a firm conclusion in this regard specifically in other crop plants also. But in this study it was not possible due to pulsity of time with limited available resources. It should be checked in future study to draw any firm conclusion.

CONCLUSION:

From the above study there is clear indication that if the isolated soil bacteria were applied either alone or in combination there will be vast improvement of plant growth and development and there will be no necessity of chemical fertilizers. So, the isolated bacteria can be used as good biofertilizers for the crop plants specially which are grown under aerially polluted zone.

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REFERENCES:

[1] Youssef MMA, Eissa MFM (2014) Biofertilizers and their role in management of plant parasitic nematodes, E J Biotechnol Pharm Res 5:1-6

[2] Hiltner L (1904) liber neuere Eefahrungen und unter besonderer Berucksichtingung der grundunging und Brache, Arb,Dtsch Landw Ges 98:59-78.

[3] Akhtar N,Qureshi MA,Iqbal A,Ahmad MJ, Khan KH (2012) Influence of Azotobacter and IAA on symbiotic performance of Rhizobium and yield paramaeters of lentil. J Agric Res 50:361-372.

[4] Ahemad M,Khan MS (2009) Effect of insecticide-tolerant and plant growth promoting Mesorhizobium on the performance of chickpea grown in insecticide stressed alluvial soils. J Crop Sci Biotechnol 12: 213-222.

[5] Berendsen RL, Pieterse CM,Bakker PAHM (2012) The rhizosphere microbiome and plant health. Trends in Plant Science 17:478-486.

[6] Lugtenberg B., Kamilova F. (2009)Plant-growth-promoting rhizobacteria. Annual Review of Microbiology 63:541-556.

[7] Van der Ent S, Van Wees SC, Pieterse CMJ (2009) Jasmonate signalling in plant interactions with resistance-inducing beneficial microbes.Phytochemistry 70:1581-1588.

[8] Adesemoye AO, Torbert HA, Kloepper JW (2008) Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system.Candian Journal Microbiology 14: 876-886.

[9] Adesemoye AO, Kloepper J(2009) Plant-microbes interactions in enhanced fertilizer-use efiiciency. Applied Microbiology and Biotechnology 14:1-12.

[10] Krupa, S.V. and Manning, W.J. (1998) Atmospheric ozone: formation and effects on vegetation. Environ. Pollut., 50, 101–137.

[11] Cho, K., Shibato, J., Agrawal, G.K., Jung, Y.H., Kubo, A., Jwa, N.S., Tamogami, S., Satoh, K., Kikuchi, S., Higashi, T., Kimura, S., Saji, H., Tanaka, Y., Iwahashi, H., Masuo, Y., and Rakwal, R. (2008) Integrated transcriptomics, proteomics, and metabolomics analyses to survey ozone responses in the leaves of rice seedlings. J. Proteome Res., 7, 2980–2998.

[12] Cappucino JG., Sherman N. (2007) Microbiology – A laboratory Manual (Ed.) Pearson Education. pp-71,145,189,146.

[13] Ashrafuzzaman M, Hossen FA, Razi Ismail M, Hoque MA, Zahurul Islam M, et al. (2009) Efficiency of plant growth-promoting rhizobacteria (PGPR) for the enhancement of rice growth. African Journal of Biotechnology 8: 1247-1252.

[14] Khalid A, Arshad M, Zahir ZA (2004) Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. Journal of Applied Microbiology 96: 473-480.

[15] Maleki M, Mostafee S, Mokhaternejad L, Farzaneh M (2010) Characterization of Pseudomonas fluorescens strain CV6 isolated from cucumber rhizosphere in Varamin as a potential biocontrol agent. Austrlian Journal of Crop Science 4: 676-683.

[16] Sandhya V, Ali SKZ, Grover M, Reddy G, Venkatswarlu B (2010) Effect of plant growth promoting Pseudomonas spp. on compatible solutes, antioxidant status and plant growth of maize under drought stress. Plant Growth Regulator 62: 21-30.

[17] Anjum MA, Sajjad MR, Akhtar N, Qureshi MA, Iqbal A, et al. (2007) Response of cotton to plant growth promoting rhizobacteria (PGPR) inoculation under different levels of nitrogen. Journal of Agricultural Research 45: 135-143.

[18] Dastager SG, Deepa CK, Pandey A (2010) Potential plant growth promoting activity of Serratianematophila NII-0.928 on black pepper (Piper nigrum L.). World Journal of Microbiology and Biotechnology 27: 259-265.

[19] Mia MAB, Shamsuddin ZH, Wahab Z, Marziah M (2010) Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and nitrogen incorporation of tissue-cultured Musa plantlets under nitrogen-free hydroponics condition. Australian Journal of Crop Science 4: 85-90.